AD_____

Award Number: DAMD17-97-1-7180

TITLE: Cell Cycle Analysis of the BRCA1 Gene Product

PRINCIPAL INVESTIGATOR: David M. Livingston, M.D.
Dennis Lynch, M.D., Ph.D.

CONTRACTING ORGANIZATION: Dana Farber Cancer Institute
Boston, Massachusetts 02115

REPORT DATE: September 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1999	Annual (15 Aug 98 -14 Aug 99)

4. TITLE AND SUBTITLE
Cell Cycle Analysis of the BRCA1 Gene Product

5. FUNDING NUMBERS
DAMD17-97-1-7180

6. AUTHOR(S)
David M. Livingston, M.D.
Dennis Lynch, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dana Farber Cancer Institute
Boston, Massachusetts 02115

E-MAIL:
david_livingston@dfci.harvard.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release;
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

please see attached.

20000530 079

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

X In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

X In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

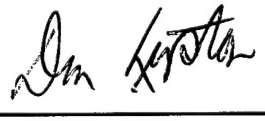

TABLE OF CONTENTS

Abstract:

During the past 12 months, our laboratory has made significant progress in the tasks of this application related to the discovery of specific BRCA1 interacting proteins. We have discovered that BRCA2 is a major BRCA1 binding protein and, based upon the data, suggested that both proteins operate together in a signaling pathway dedicated to DNA damage/genome integrity control. In addition, we have accumulated data which suggest that the function of this pathway is linked to the suppression of breast and ovarian cancer. In more recent experiments, we have discovered a novel protein (p130) that specifically interacts, in vivo, with the BRCT domains of BRCA1. We have cloned this polypeptide and deciphered its amino acid sequence, which indicates that its product is a new member of a family of DNA-dependent helicases. In this regard, it is clearly related the ERCC4 (XP-D) class of nucleotide excision repair proteins. We are now engaged in experiments aimed at deciphering the biochemical and,ideally, the biological implications of this interaction, which can now be readily detected in untransfected human cells.

5. Introduction: The goal of this research is to understand how BRCA1 functions in biochemical terms. The primary specific aim of the project was to search for specific proteins with which BRCA1 might interact. From much existing evidence, there is reason to hypothesize that a significant fraction of the function of BRCA1 operates through interactions with specific nuclear proteins. The work of the past year was again aimed at searching for new BRCA1 interacting proteins in human cells.

6. BODY : As described in the appended publication (Chen et al, Mol Cell 1998;2:317-328), we succeeded in identifying BRCA2 as a physiological binding partner of BRCA1. The data herein show that the two proteins interact through a specific C-terminal segment of BRCA1 and, although both also interact with Rad 51, it is not the linking unit. The proteins co-IP from extracts of various, naïve human cell lines using monospecific Abs to BRCA1, BRCA2, and/or Rad 51. They also coexist in S/G2 phase nuclear dots and migrate to PCNA-containing replication foci after S phase DNA damage. Finally, they coexist on the unsynapsed portions of developing human synaptanemal complexes. Since BRCA2, like BRCA1, is a suppressor of early onset breast and ovarian cancer, these data suggest that the BRCA1/2 complex, which also contains BARD1 and Rad 51, plays a critical role in the operation of a DNA damage response pathway dedicated, at least in part, to breast and ovarian cancer cell development.

More recently, we have begun to search for proteins that interact specifically with the BRCT repeat motifs of BRCA1. These C-terminal structures operate as i) critical contributors to BRCA1 tumor suppression function; ii) the basis for BRCA1 transcription activation function and binding sites for RNA polymerase holoenzyme; and iii) entry sequences for the protein into nuclear dot structures (Chen, J. and Livingston, D. unpublished). In order to identify proteins that bind to the BRCT repeats of BRCA1, we generated BRCT repeats fused to GST. After immunoprecipitation of endogenous BRCA1, Far Western experiments using the BRCT-GST protein as a probe, showed that at least four proteins bound to BRCA1. These four proteins did not bind the BRCT-GST probe when clinically relevant mutations were made. After failing to detect these proteins in expression libraries, we were able to use the BRCT-GST fusion as an affinity column to capture one of the four bands first identified in the BRCA1 immunoprecipitation. This band is 130 kd.

We have purified enough of this protein from gels to obtain limited polypeptide sequence and have now cloned and sequenced its cDNA. From its imputed polypeptide sequence, the clonal protein appears to be anATP-dependent DNA helicase involved in excision repair with homology to ERCC2. Clearly, there is much more to do, but the data, thus far, point to the identification of a new, interesting physiological BRCA1 binding protein and, therefore, suggest that we now face an opportunity to extend our understanding of how BRCA1 operates, biochemically.

In addition to completing the cloning and sequencing of a full length p130 cDNA, we are now trying to generate monospecific Abs to it and to use these reagents for a number of future studies including a test of its ability to interact with a series of clinically relevant mutant forms of BRCA1.

7. Key Research Accomplishments

- Discovery of a physiological interaction of BRCA1 and 2.
- Discovery and cloning of a new, BRCA1- associated protein, p130, which specifically interacts with the BRCT motifs of the protein.

8. Reportable Outcomes

Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Minna JD, Couch FJ, Weber BL, Ashley T, Livingston DM, Scully R. Stable interaction the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Mol Cell 1998; 2:317-328.

Chen, J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Minna JD Couch FJ, Weber BL, Ashley T, Livingston DM, Scully R. The products of the RCA1 and BRCA2 tumor suppressor genes interact stably in mitotic and meiotic cells. CSH Meeting on Cancer Genetics and Tumor Suppressor Genes, Cold Spring Harbor, NY, Aug 19-23, 1998.

Livingston DM. Invited Speaker, Columbia University College of Physician and Surgeons, December 17, 1998: "Functional Analysis of the BRCA1 and 2 Tumor Suppressor Gene Products."

Livingston*, DM, Cantor, S, Chen J, Joukov V, Scully R, Liver D, Wu X. "Insights into the molecular basis for BRCA1 and BRCA2 Function," The Genetics Society of America Meeting on "DNA Repair: Bacteria to Humans," Arlie House Conference Center, Warrenton, VA, April 16-19, 1998.
*speaker

Livingston*, DM, Chen J, Scully S, Silver DP, Cantor S, Joukov, V. "Functional analysis of the BRCA1 and BRCA2 tumor suppressor gene products." The American Society of Hematology Annual Meeting, Miami Beach, Fl, Dec 4-8, 1998.
*speaker

9. Conclusions: The accomplishments noted above have advanced our understanding of how BRCA1 functions. Specifically, they point, for the first time, to a molecular explanation for why the clinical outcomes of BRCA1 and 2 disease are so similar. They also point to the existence of a coordinated signaling pathway in which both of the known, inherited breast/ovarian cancer gene products (BRCA1 and 2) operate. They also suggest that such a pathway is dedicated to genome integrity control and to the specific suppression of breast and ovarian cancer.

The discovery of p130 offers a new opportunity to probe the biochemical function of BRCA1, to understand the biochemical mechanisms which underlie the physiological operation of its BRCT motifs, and, possibly, to begin to understand how its biochemical function(s) translates into its tumor suppression function.

10. references

Scully R, Chen J, Ochs RL, Keegan K, Hoeckstra M, Feunteun J, Livingston DM. Dynamic changes during the S phase of BRCA1 subnuclear localization and phosphorylation state are initiated by DNA damage. Cell 1997;90:425-435.

Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G, Minna JD, Couch FJ, Weber BL, Ashley T, Livingston DM, Scully R. Stable interaction the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. Mol Cell 1998; 2:317-328.